Atlantic Zonal Monitoring Program Sampling Protocol

Michel R. Mitchell, Glen Harrison, Kevin Pauley
Alain Gagné. Gary Maillet, and Peter Strain

Ocean Sciences Division
Maritimes Region
Fisheries and Oceans Canada

Bedford Institute of Oceanography
P.O. Box 1006
Dartmouth, Nova Scotia
Canada B2Y 4A2

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Michel R. Mitchell, Glen Harrison, Kevin Pauley,
Alain Gagné[1], Gary Maillet[2], and Peter Strain[3]

Ocean Sciences Division
Maritimes Region
Fisheries and Oceans Canada
Bedford Institute of Oceanography
P. O. Box 1006
Dartmouth, Nova Scotia
Canada, B2Y 4A2

1. Ocean Science Direction, Québec Region, Fisheries and Oceans Canada, Institut Maurice Lamontagne , 850 route de la Mer, C.P. 1000, Mont-Joli, Québec, Canada, G5H 3Z4

2. Aquatic Resources Division, Newfoundland Region, Fisheries and Oceans Canada, Northwest Atlantic Fisheries Centre , P.O. Box 5667, St. John's, Newfoundland, Canada, A1C 5X1

3. Marine Environmental Sciences Division, Maritimes Region, Fisheries and Oceans Canada, Bedford Institute of Oceanography, P. O. Box 1006, Dartmouth, Nova Scotia, Canada, B2Y 4A2

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ABSTRACT

Mitchell, M.R., G. Harrison, K. Pauley, A. Gagné, G. Maillet, and P. Strain. 2002. Atlantic Zonal Monitoring Program Sampling Protocol. Can. Tech. Rep. Hydrogr. Ocean Sci. 223: iv + 23 pp.

This report describes the sampling protocols for the Atlantic Zonal Monitoring Program (AZMP). These protocols are used by field personnel from the various regional laboratories involved in the AZMP to ensure a consistent approach to the collection of data throughout the zone. The report, which is largely a guide for field personnel, summarises the sampling operations and outlines the general procedures needed to acquire the basic AZMP data.

RÉSUMÉ

Mitchell, M.R., G. Harrison, K. Pauley, A. Gagné, G. Maillet, and P. Strain. 2002. Atlantic Zonal Monitoring Program Sampling Protocol. Can. Tech. Rep. Hydrogr. Ocean Sci. 223: iv + 23 pp.

Le présent rapport décrit les protocoles d'échantillonnage du Programme de monitorage de la zone Atlantique (PMZA). Ces protocoles sont utilisés par le personnel de terrain des divers laboratoires régionaux participant au PMZA. Ils visent à faire en sorte que la collecte de données s'effectue de manière homogène dans toute la zone. Le rapport, qui est en grande partie un guide pour le personnel de terrain, résume les opérations d'échantillonnage et décrit les procédures générales nécessaires pour acquérir les données fondamentales du PMZA.

1 Introduction

The field component of the Atlantic Zone Monitoring Program (AZMP) aims to collect selected data in order to detect and monitor seasonal and interannual variability of biological, chemical and physical properties of the coastal waters of eastern Canada. Therriault et al. (1998) describe the program, its goals and its design in detail.

This document presents the standard protocols that are to be used in all regions contributing data for the AZMP in order to ensure a consistent approach to the collection of the data throughout the zone. The general procedures outlined below are well established and widely-used by the oceanographic community and may differ among the various laboratories by only minor modifications. These protocols are largely a guide for the sampling and analysis. Nevertheless, the AZMP identifies a specific array of variables that must be measured as described in the required sampling below.

2 Required sampling

The AZMP requires sampling monthly or more frequently at fixed sites and semi-annually along transects.

At the fixed stations, the minimum sampling operation includes:

1. Vertical profile of the entire water column using a CTD equipped with at least a fluorometer
2. Water bottle sampling at selected depths for:
   - i. nutrients measurement – duplicates at all depths
   - ii. chlorophyll-*a* extraction – duplicates at all depths
   - iii. phytoplankton cell counts – 1 full water column sample pooled from each individual bottle
   - iv. dissolved oxygen – duplicates at surface and bottom
   - v. salinity measurement – surface and bottom
3. Vertical net tows for zooplankton
4. Secchi depth measurement

At stations along the transects, the minimum sampling operation includes:

1. Vertical profile of the entire water column using a CTD equipped with at least a fluorometer
2. Water bottle sampling at selected depths for :
   - i. nutrients measurement – duplicates at all depths
   - ii. chlorophyll-*a* extraction – duplicates at all depths
   - iii. dissolved oxygen – duplicates at surface and bottom
   - iv. salinity measurement – surface and bottom; subset of 10 or more CTD stations
3. Vertical net tows for zooplankton
4. Secchi depth measurement

The number of water bottle depths is determined by the station characteristics. Typically, there will be 6 to 10 bottles at each station, including a surface and a bottom sample, and sufficient samples in between to properly calibrate the CTD fluorometer and oxygen sensor.

3 Sampling procedures

3.1 CTD Profile

A CTD cast from the surface to the bottom is executed at all stations. A fluorometer should be included in the CTD instrumentation. An oxygen probe is also highly desirable. If the fluorometer used does not permit deep casts, two CTD profiles are required: one profile with the fluorometer down to the design depth of the instrument, and one full depth profile without the fluorometer.

Prior to deployment, all relevant information, including the serial number of the instruments, should be recorded on a hard copy logsheet. Also prior to each deployment, the CTD sensors should be checked to ensure that the sensors are clean and free of any fouling and that all protective coverings and tubing have been removed. All system battery supplies should be switched on and the instruments should be switched on while still on deck. All the values displayed by the sensors should be observed to ensure the system is functioning properly. The value displayed by the pressure and temperature sensors while the CTD is still on deck should be recorded on the log sheet. This can be used to correct the logged pressure.

On CTD rosette systems, check that Niskin bottles are correctly set and taps and vents are closed.

The CTD is deployed and allowed to sit at the surface for a minimum of 3 minutes to allow the pump system to turn on and all the sensors to stabilize. Record the current time and current depth sounding on the logsheet. The CTD is then lowered continuously at a targeted speed of 1 meter/sec to the maximum depth for the station. Any water samples collected with a rosette system are collected while the CTD is returning to the surface. Usually the CTD is lowered to within 3 to 10 metres from the bottom. How close to the bottom the CTD is actually lowered will depend on the quality of the sounding, weather conditions, sea conditions, bottom topography and whether the system is equipped with additional bottom detection and avoidance equipment (e.g. altimeter).

When the CTD is at the bottom of the cast, it should remain at the bottom for at least one minute for the sensors to reach equilibrium.

On the up cast, water samples are collected by stopping the ascent at selected depths.

When the CTD is brought out of the water, record any unusual observation on the conditions of the instrument package. When the unit is onboard, plug the sensor duct and rinse with fresh water.

3.2 Water samples

Plots from the CTD profile are examined to determine the depth of the chlorophyll-*a* maximum. Water samples are collected from sufficient depths (usually 6 depths) within the euphotic zone, including one at the chlorophyll-*a* maximum, to properly calibrate the CTD fluorometer; and from the surface and bottom of the water column. Water samples are collected using conventionnal sampling equipment (e.g. manually deployed Niskin-type bottles on a hydrowire; rosette-mounted sampling bottles) and stored in appropriate containers. The volumes of water required for each sample, excluding amounts wasted for rinsing, are as follows:

a) Two 350 ml samples at the surface and at the bottom for oxygen;

b) Two 30 ml samples at each depth for nutrients;

c) Two 100 ml samples at each depth for chlorophyll-*a;*

d) One 100 ml sample from each depth at the fixed stations to be mixed together to produce one large volume from which a 500 ml subsample is taken for phytoplankton cell counts.

e) One 200 ml sample for salinity at the surface and at the bottom

Samples should always be drawn in the same order from the bottle: dissolved oxygen first, nutrients next, and then other samples. All samples should be properly labeled: all samples from a same bottle should use the same label number.

Water for the oxygen samples is drawn directly from the sampler bottle and before any of the other samples. Water for nutrients, chlorophyll-*a* and phytoplankton cell counts can be collected in a common properly cleaned and rinsed container for subsampling elsewhere as soon as possible.

The volume of water for chlorophyll-*a* analysis may be more than 100 ml when required. This should be clearly recorded on the log sheets.

3.3 Dissolved Oxygen

Samples are taken in duplicates with identical labels.

3.3.1 Samples to be analyzed by Winkler Titration

For samples that will be analyzed by the Winkler titration method, the collection of oxygen samples is a two step process: drawing the sample from the sampler and pickling the sample.

Drawing the sample:

1) Check to ensure the flask and finger cap you are about to use are inscribed with the same number.
2) Attach a silicone tube to the sample device (Niskin bottle) outlet, open the vent and bleed water through the tube to expel any air bubbles.
3) Pinch the tube and insert its end into the flask, invert the flask and allow water to flow up into it. Swirl the flask until you can feel its temperature is the same as the water. Allow wastewater to run over the flask finger.
4) Reorient the flask, slowly pinching the tube to minimize splashing and air bubble entrapment as the flask fills.
5) Fill the flask to three flask volumes (allow the flask to overfill: count the number of seconds for the initial fill and multiply by three) keeping the fill tube near the bottom of the flask.
6) Slowly remove the tube, pinching off the flow to minimize splashing.
7) Immediately pickle the sample as described below.

Pickling the sample:

8) Check to ensure there are no bubbles in the reagent pipettor line and ensure that the dispensing volume is set to 1.00 ml for each reagent (or, if corrected, at the appropriate mark)
9) Raise the pipettor plunger, then immerse the pipettor tip into the filled flask and SLOWLY inject first the alkaline, then the Manganous Chloride reagents. The object is to introduce the reagents near the bottom of the flask; being very dense they will sink and mix from the bottom up. The overlying surface water in the flask would be reagent free and discarded when the finger is inserted.
10) Reinsert the finger, ensuring no air bubbles are trapped in the flask, and then shake vigorously.
11) Fill cupped top of flask with super-Q water. This minimizes any changes of exchange with atmospheric oxygen and serves to clean the cup.
12) The samples are stored at room temperature, out of direct light.

3.3.2 Samples to be analyzed by dissolved oxygen meter

The oxygen samples intended for measurement with dissolved oxygen meter require no pickling. BOD bottles are used. The samples are drawn as follows:

1) Attach a silicone tube to the sample device (Niskin bottle) outlet, open the vent and bleed water through the tube to expel any air bubbles.
2) Pinch the tube and insert its end into the bottle, invert the bottle and allow water to flow up into it. Swirl the bottle until you can feel its temperature is the same as the water. Allow wastewater to run over the bottle closure.
3) Reorient the bottle, slowly pinching the tube to minimize splashing and air bubble entrapment as the bottle fills.
4) Fill the bottle to three bottle volumes (allow the bottle to overfill: count the number of seconds for the initial fill and multiply by three) keeping the fill tube near the bottom of the bottle.
5) Slowly remove the tube, pinching off the flow to minimize splashing.
6) Reinsert the closure, ensuring no air bubbles are trapped in the bottle.
7) The samples are stored at room temperature, out of direct light. Dissolved oxygen should be measured with the portable dissolved oxygen meter as soon as possible.

3.4 Nutrients

In preparation, the bottles intended for nutrient samples (NO_3, PO_4, SiO_3) should have been Acid washed (10% HCl), rinsed 3 times with deionized water and dried before capping.

Samples are taken in duplicates with identical labels in 30-ml HDPE bottles.

To sample, open the spigot on the sampler, remove the caps from duplicate bottles and rinse both bottles and caps three times before filling to the neck. Do not fill the bottle to the top, as room is required for expansion during freezing. Cap the bottles and, as soon as possible, place the filled bottles right side up in a freezer for long term storage. If filling the nutrients bottles from a common container, this container is also rinsed three times, and the above rinsing, filling and storing protocol applies to the nutrient bottles.

In coastal waters with significant sediment load, it may be necessary to filter the samples prior to nutrient analysis. If filtering is not necessary, then it should be avoided to minimize sample contamination.

Precautions: special care is needed to avoid finger contamination of the nutrient sample, as PO_4 is highly sensitive to such contamination.

3.5 Chlorophyll-a

Two 100 ml aliquots are drawn from each water bottle and filtered via vacuum filtration onto 25 mm glass fibre filters (GFF). After the filtration the glass fiber filters are deposited into separate scintillation vials containing 10 ml of 90% acetone. Caps must be sealed tightly to ensure no leakage or evaporation of the acetone. The vials are identically labeled, and stored in an explosion-proof freezer for eventual chlorophyll-*a* extraction.

3.6 Phytoplankton cell counts

One 100-ml aliquot will be drawn from each of the sampler bottles (collected from the surface to the bottom of the water column) and combined into a single container for thorough mixing. A well-mixed 500-ml subsample will then be drawn from this pooled sample and preserved in a labeled sample container with 2% Lugol's preservative (see sample preparation in appendix III). The container is labeled with an identifier label from each of the bottles contributing to the integrated sample.

3.7 Salinity

One 200-ml aliquot is drawn. The sample bottle is first labeled and then rinsed twice. To rinse, fill bottle to 1/3, recap, shake well and empty the bottle. Only fill the bottle to the base of the neck: space is required for volume expansion as cold samples warm up.

3.8 Zooplankton

The following standard protocols are to be used for routine sampling of zooplankton at fixed and transect stations. At all stations, at least one standard zooplankton vertical tow with 202-µm mesh net is taken.

At the time of capture, gelatinous zooplankton are removed from the catch, identified according to major taxonomic category (e.g. siphonophore, ctenophore, medusae), measured volumetrically and a sub-sample of this gelatinous zooplankton catch is preserved separately for confirmation of identification. The remainder of the sample is preserved in a 4% solution of buffered formaldehyde.

STANDARD ZOOPLANKTON TOW

NET TYPE: 3/4 m ring net
MESH SIZE: 202 µm
TOW METHOD: vertical (see note 1)
DEPTH: bottom-surface or 1000-0 m, whichever is shallower.
REPLICATION: see note 2

Note 1:
The vertical net is installed on the wire with a cross-bow support. Where possible, flow meters are installed for comparison of volume calculated from tow depth and net area; weather and sampling conditions that may potentially cause discrepancies between these two methods should be noted. During deployment, the ship maneuvers to maintain vertical wire angle. The targeted tow speed is 1 m s-1.

Note 2:
Normally one tow per station. At analysis time, the sample is split for biomass estimates and for zooplankton species abundance.

3.9 Secchi depth

A white Secchi disk is lowered vertically and the greatest depth at which it can be visually detected is recorded.

4 Analysis

4.1 Dissolved Oxygen

Oxygen will be analyzed using the well-known Winkler titration method. Samples can be processed either manually or using a semi-automated micro-processor controlled titration apparatus (e.g.. Strain and Clement, 1996).

Measurements obtained using a suitable portable dissolved oxygen meter (e.g. Orion model 835A, with polarographic electrode probe) are also acceptable. The accuracy of the Orion model 835A is reported as ± 0.5% and the meter features automatic barometric pressure and temperature compensation. Field measurements obtained with this model have been compared to Winkler titration results and found to be very reliable (Peter Strain, personnal communications). To ensure quality data the meter must be properly standardized before each set and the probe properly rinsed with deionized water between each sample. Special care must be given to prevent air bubbles being trapped at the probe membrane interface while inserting the probe into the sample bottle.

4.2 Nutrients

Nutrients (nitrate, phosphate, silicate) will be analyzed using established colorimetric techniques using a segmented-flow autoanalyzer (e.g. Technicon AutoAnalyzer II). The details of the instrument setup, chemistry, and calibration are provided in Appendix I (extracted from Strain and Clement, 1996).

4.3 *Chlorophyll-a*

Chlorophyll-*a* will be determined on acetone extracted samples using a modification of the fluorometric procedure first described by Holm-Hansen et al. (1965). An outline of the procedure is given in Appendix II. The Québec region also employs a slightly modified version of this method to correct for interfering pigments (Welschmeyer, 1994).

4.4 Phytoplankton cell counts

Phytoplankton cells are counted and identified using Utermöhl (1931) settling chambers, or a derivation there-of, and a phase contrast microscope. A 10-ml subsample of the preserved sample is allowed to deposit as sediment in 10 ml counting chambers directly onto glass microscope slides. The preserved samples are analyzed for identification and enumeration to both the genus and species levels, considering the 20 most dominant species. Enumeration results are pooled into taxonomic categories such as diatoms, both centric and pennate, dinoflagellates and flagellate / ciliates for the purposes of examining community structure. The method is described in more details is appendix III.

Additional subsamples of the collections can be forwarded to other laboratories for an intercomparison analysis.

4.5 Zooplankton

The preserved samples are analyzed for biomass (dry wt m^{-2}) and species composition and abundance. After analysis a portion of each preserved sample will be archived at a regional center.

Accurately separating samples into size fractions has frequently proven to be very difficult because of the high concentrations of phytoplankton, appendicularians, jellies, salps, etc. This results in unreliable measurements. Therefore in order to reduce the difficulty of separating large and small plankton for biomass measurements, the following protocol is to be used. After pouring off the formalin, all organisms larger than 1 cm are manually separated out. These organisms are identified according to the criteria listed in appendix V (as for small organisms) and weighed (wet weights, which permits these organisms to be preserved). This total weight is reported. The remainder of the sample (i.e. all organisms less than 1 cm) is split once using a Matoda splitter. One half of the sample is used for dry weight where the animals are collected on a pre-weighed shark skin filter, dried at 60°C for 48 hours and weighed. The other half of the sample is used for abundance/composition determinations as detailed in appendix V.

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Appendix I. Nutrient Analyses

All nutrient analyses are performed using colorimetric techniques on a Technicon AutoAnalyzer II (AA II) segmented flow analyzer. Calibrations are done using a series of standards at six different concentrations analyzed at the beginning and end of each AA II run. All standards, and most samples, are analyzed in duplicate. Duplicate check standards, followed by duplicate blanks, are interspersed through the run (usually at intervals of sixteen samples). Typical runs consist of 150 samples, standards and blanks run at 30 samples/hr using equal length sample and wash cycles. For silicate, phosphate, nitrate, and nitrite, standards are prepared in NaCl solution (33 g/L); the latter is also used for wash water. For ammonia, freshwater standards and wash water are used. Detection limits and analytical precisions are determined for each nutrient for each run: detection limits are equal to three times the standard deviation of the blanks; the precision estimates are equal to the standard deviation of the check standards. The precision of the mean value for most samples (most samples are analyzed in duplicate) will be this precision divided by $\sqrt{2}$. These measures of analytical performance depend on the nature and source of the samples and the concentration range of the calibration standards as well as on the performance of the analytical equipment.

Because no certified reference materials are available for nutrients in seawater, it is not possible to determine analytical accuracy in a rigorous way. However, CSK standards (from the Sagami Research Center, Japan) are available. As these standards are prepared either in NaCl solution or in freshwater, they are not ideal reference materials. However, our experience has been that they are reasonably stable and useful for silicate, nitrate, and nitrite (nitrite CSK standards are not run frequently). CSK's are also available for phosphate, but erratic results and short shelf life make their use problematic. As an alternative approach to evaluating accuracy, our laboratory participates in the regular seawater nutrient intercalibration exercises organized by the International Council for the Exploration of the Seas. We have produced results consistent with the final 'accepted' values for silicate, phosphate, and nitrate (e.g. Kirkwood et al. 1991, in which we were lab number 72). To date, we have not participated in the nitrite and ammonia portions of these intercalibrations because nitrite is an analysis we only do in special circumstances and the ammonia method we use is only suitable for the high concentrations found near shore (see below).

More details on the methods for the individual nutrients follow.

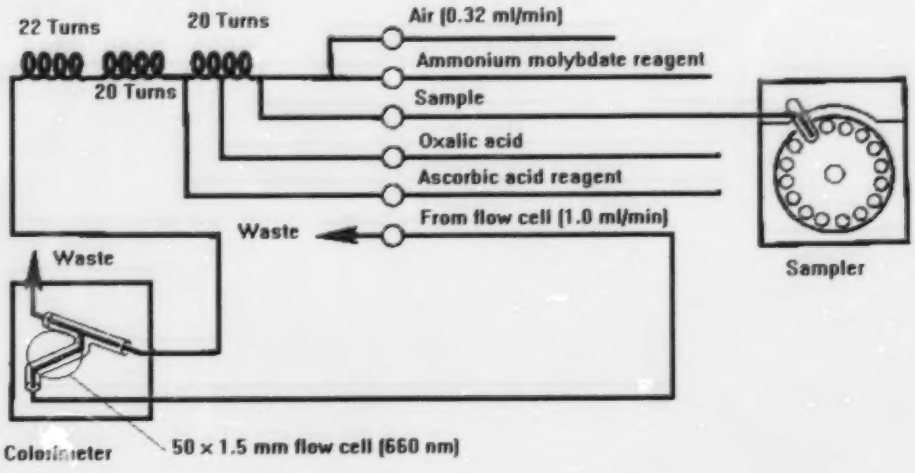
Silicate

The analysis of reactive silicate is based on the formation of silicomolybdic acid and its subsequent reduction to a blue heteropoly acid. The basic chemistry is described in Grasshoff, 1976 (and references therein). These samples were analyzed using the implementation of the method developed by Technicon (Technicon Industrial Method 186-72 W, 1973; see reagent table and flow diagram below). The main difference between this method and earlier ones is its use of ascorbic acid as a reducing agent. The rms differences between expected and nominal concentrations of silicate for CSK standards are 5.7, 3.6 and 2.5 % at concentrations of 5, 25 and 50 µM, respectively.

Table 1. Reagents used in silicate analysis.

Reagent	Flow rate (ml/min)	Component	Concentration
Ammonium molybdate reagent	0.42	$(NH_4)_6Mo_7O_{24}•4H_2O$ H_2SO_4	10 g/L 0.1 N
Oxalic acid	0.32	$H_2C_2O_4$	50 g/L
Ascorbic acid reagent	0.42	Ascorbic acid Acetone Levor V	17.6 g/L 50 ml/L 0.5 ml/L
Sample	0.32		

Figure 1. Flow diagram for silicate analysis.



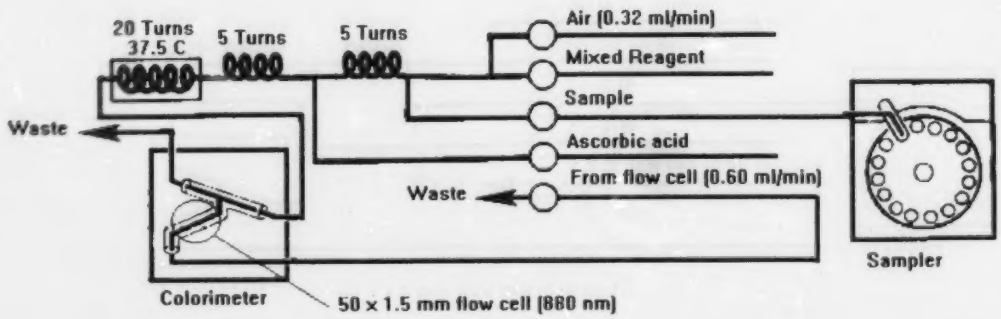
Phosphate

The analysis of dissolved inorganic phosphate is based on the formation of a phosphomolybdenum blue complex, following a method originally described by Murphy and Riley (1962). We use a slightly modified version of the implementation for autoanalyzers described in Technicon Industrial Method 155-71W, 1973. Our modification is one that is widely used (see Kirkwood et al., 1991). The mixed reagent is separated into two components to improve reagent stability, but the chemistry of the final mixture of sample and reagents in the spectrometer cell has not been altered.

Table 2. Reagents used in phosphate analysis.

Reagent	Flow rate (ml/min)	Component	Concentration
Mixed reagent	0.23	H_2SO_4 $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ $K(SbO)C_4H_4O_6{\cdot}½H_2O$ (antimony potassium tartrate)	2.45 N 6 g/L 0.15 g/L
Ascorbic acid	0.32	Ascorbic acid Levor V	3.9 g/L 2 ml/L
Sample	0.42		

Figure 2. Flow diagram for phosphate analysis.



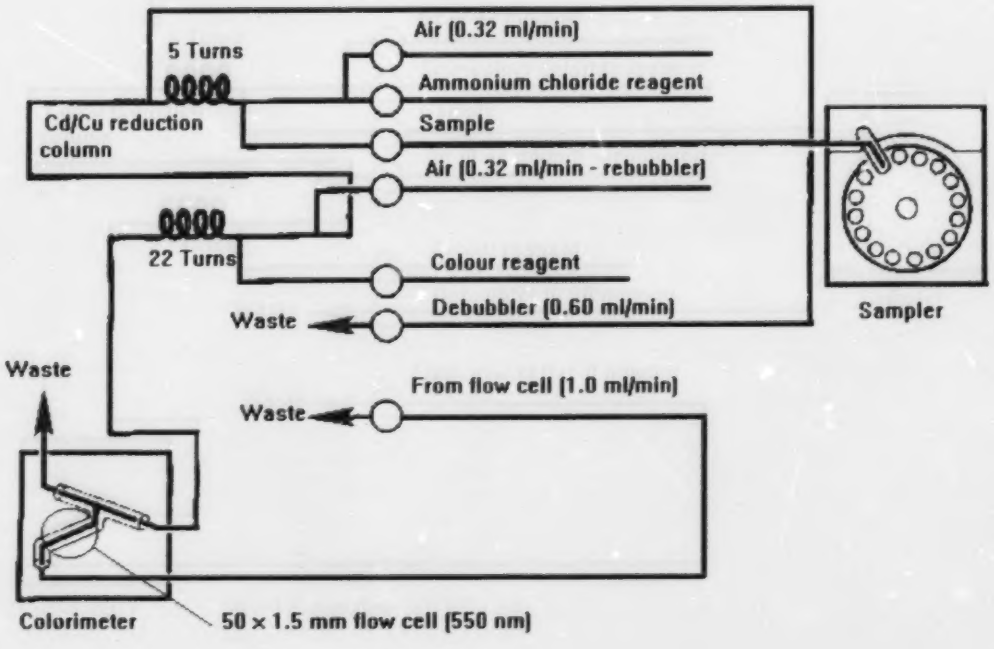
Nitrate

The analysis of nitrate is based on the measurement of a diazo dye formed by the reaction between sulfanilamide and nitrite, which in turn has been produced by the reduction of nitrate to nitrite on a copperized cadmium column. Because the analysis is based on the reduction of nitrate to nitrite, the method actually determines the sum of the nitrate and nitrite concentrations. In most seawater, the concentration of nitrite is small compared to that of nitrate. The basic chemistry is described by Grasshoff, 1976. We use the implementation of the method described in Technicon Industrial Method 158-71W, 1972. The rms differences between expected and nominal concentrations of nitrate for CSK standards are 3.1, 1.7 and 1.8 % at concentrations of 5, 10 and 30 µM, respectively.

Table 3. Reagents used in nitrate analysis.

Reagent	Flow rate (ml/min)	Component	Concentration
Ammonium chloride reagent	1.2	NH_4Cl NaOH	10 g/L 0.6 g/L
Colour reagent	0.32	Sulphanilamide H_3PO_4 (conc.) N-1-Napthylene-diamine dihydrochloride Brij-35	10 g/L 100 ml/L 0.5 g/L 0.5 ml/L
Copperizing reagent	N/A	$CuSO_4 \cdot 5H_2O$	20 g/L
Sample	0.32		

Figure 3. Flow diagram for nitrate analysis.



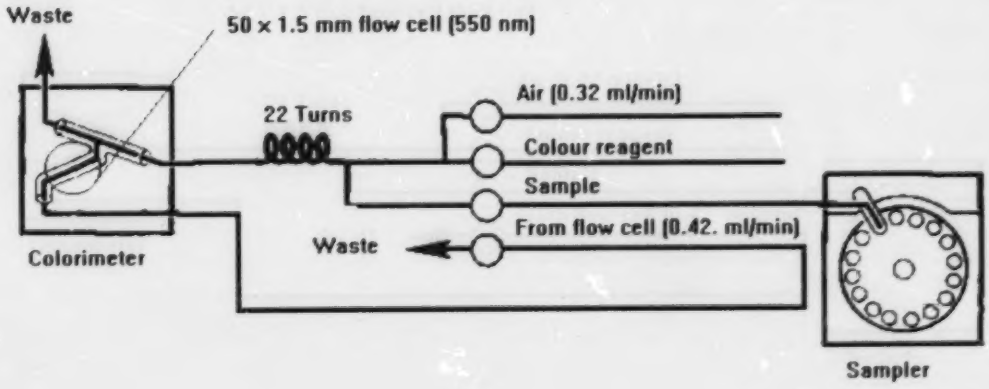
Nitrite

The analysis of nitrite is essentially the same as that for nitrate, but without the cadmium column reduction step. We use the implementation of the method described in Technicon Industrial Method 161-71W, 1973. The rms differences between expected and nominal concentrations for a recent set of nitrite CSK standards were 2.2, 1.3 and 5.4 % at concentrations of 0.5, 1.0 and 2.0 µM, respectively.

Table 4. Reagents used in nitrite analysis.

Reagent	Flow rate (ml/min)	Component	Concentration
Colour reagent	0.6	Sulphanilamide	10 g/L
		H_3PO_4 (conc.)	100 ml/L
		N-1-Napthylene-diamine dihydrochloride	0.5 g/L
		Brij-35	0.5 ml/L
Sample	0.6		

Figure 4. Flow diagram for nitrite analysis.



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Appendix II. Measurement of Chlorophyll-*a* and Phaeopigments by Fluorometric Analysis

Scope and field of application

Chlorophyll-*a* measurements have historically provided a useful estimate of algal biomass and its spatial and temporal variability. The fluorometric method (Holm-Hansen et al. 1965) is extensively used for the quantitative analysis of chlorophyll-*a* and phaeopigments. The procedure described here is appropriate for all levels of chlorophyll-*a* concentration in the marine environment. Filtration volumes should be modified for the different environments. Scientists who employ this or other methods to measure pigments should make themselves aware of the current and historical issues that surround these techniques.

Definition

The concentrations of chlorophyll-*a* and phaeopigments in seawater are given as µg/L.

Principle of Analysis

Algal pigments, particularly chlorophyll-*a*, fluoresce in the red wavelengths after extraction in acetone when they are excited by blue wavelengths of light. The fluorometer excites the extracted sample with a broadband blue light and the resulting fluorescence in the red is detected by a photomultiplier. The significant fluorescence by phaeopigments is corrected for by acidifying the sample which converts all of the chlorophyll-*a* to phaeopigments. By applying a measured conversion for the relative strength of chlorophyll and phaeopigment fluorescence, the two values can be used to calculate both the chlorophyll-*a* and phaeopigment concentrations.

Apparatus

1. Filtration system and 25 mm diameter glass fibre filters (Whatman GF/F or equivalent)

2. Liquid nitrogen and freezer (-20C) for storage and pigment extraction

3. Turner fluorometer, fitted with a red sensitive photomultiplier, a blue lamp, 5-60 blue filter and 2-64 red filter.

Reagents

1. 90% acetone

2. 1.2M HCl (100 ml HCl in 900 ml de-ionized water)

Sample Collection and Storage

Water samples are collected from Niskins into clean polyethylene bottles with Tygon® or silicone tubing. Samples (generally 100 ml, in duplicates) are immediately filtered through 25 mm glass-fibre filters under a vacuum of less than 100 mm Hg. Filters are then immersed in 10 ml of 90% acetone contained in 20 ml glass scintillation vials, the vials are then tightly capped (caps should have plastic liners) and stored in a freezer (-20 C) for 24 h for complete pigment extraction. Alternatively, after sample filtration, filters are folded in half and wrapped in aluminum foil, labeled, and stored in liquid nitrogen (to avoid formation of degradation products) until analysed later.

Procedure

1. After removal from liquid nitrogen, the pigments are extracted from the stored filters as described above. All sample processing should be done in subdued light.

2. The fluorometer is allowed to warm up and stabilize for 30 minutes prior to use.

3. The fluorometer is zeroed with 90% acetone or an acetone "blank" is determined.

4. Following extraction, samples are allowed to warm to room temperature in the dark and are decanted into a cuvette and read on the appropriate scale (sensitivity) setting. The sample is then acidified with 2 drops of 1.2 M HCl and read again. Dilutions may be necessary for high chlorophyll-*a* concentrations samples.

Standardization

1. For laboratory use, the fluorometer is calibrated every 6 months with a commercially available chlorophyll-*a* standard (*Anacystis nidulans*, Sigma Chemical Company). If the fluorometer is taken to sea, it is recommended that the fluorometer be calibrated before and after each cruise.

2. The standard is dissolved in 90% acetone for at least 24 h and it's concentration (mg/L) is calculated spectrophotometrically as follows;

$$\text{Chl } a = \frac{(A_{max} - A_{750\,nm})}{Exl} x \frac{1000mg}{1gram}$$

where:

A_{max} = absorption maximum (664 nm)

$A_{750\,nm}$ = absorbance at 750 nm to correct for light scattering

E = extinction coefficient for chl *a* in 90% acetone at 664 nm (87.67 L g^{-1} cm^{-1})

l = cuvette path length (cm)

3. From the standard, a minimum of five dilutions are prepared for each scale setting. Fluorometer readings are taken before and after acidification with 2 drops 1.2 M HCl.

4. Linear calibration factors (K_x) are calculated for each scale (x) as the slope of the unacidified fluorometric reading vs. chlorophyll-*a* concentration calculated spectrophotometrically.

5. The acidification coefficient (F_m) is calculated by averaging the ratio of the unacidified and acidified readings (F_o/F_z) of pure chlorophyll-*a*.

6. Samples are read using a scale setting that produces a dial reading between 30 and 90. The fluorometer is zeroed with 90% acetone each time the scale setting is changed or an acetone "blank" is determined.

Calculation and expression of results

The concentrations of chlorophyll-*a* and phaeopigments in the sample are calculated using the following equations:

$$\text{Chl } a \text{ (ug/L)} = \left(\frac{F_m}{F_m - 1}\right) \times (F_o - F_a) \times K_x \times \left(\frac{vol_{ex}}{vol_{filt}}\right)$$

$$\text{Phaeo (ug/L)} = \left(\frac{F_m}{F_m - 1}\right) \times [(F_m \times F_a) - F_o] \times K_x \times \left(\frac{vol_{ex}}{vol_{filt}}\right)$$

where:

F_m = acidification coefficient (F_o/F_a) for pure Chl *a* (usually 2.2).

F_o = reading before acidification

F_a = reading after acidification

K_x = scale factor from calibration calculations

vol_{ex} = extraction volume

vol_{filt} = sample volume

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Appendix III. Phytoplankton Sampling and Analysis

The samples are collected from fixed stations using Niskin bottles. Each sample is taken from a discrete depth and is identified by a unique identifier label. The multi-depth sampling design allows subsamples from all depth to be pooled to yield an integrated subsample of the water column. Subsamples of 100 mL are withdrawn from each sample depth collection and placed in a single container. A 250-mL subsample is then removed from this container and placed in an opaque plastic container bearing all of the unique sample labels, indicating the source of the subsample donation. The integration of the water column samples is done in an effort to resolve the patchiness often associated with vertical phytoplankton distributions (Sournia, 1978). In instances where the fluorometric profile of the water column indicates a distinct chlorophyll-*a* maximum a subsample of 20 mL is obtained from the Niskin bottle and placed in a glass scintillation vial labelled with the corresponding water depth label. This discrete sample would allow the identification of the specific phytoplankton taxon contributing to the fluorescence peak and will allow some comparison to the integrated sample taken at the same time.

Sample Preservation

Lugol's iodine, also known as acid Lugol's was chosen as a preservative for the phytoplankton sample preservation in this study for a number of reasons. The preservative can be purchased or made by mixing (200 g potassium iodide, 100 g crystalline iodide, 2000 mL of distilled water and 190 mL glacial acetic acid) as described in Sournia (1978) and Parsons et al. (1984). Primarily this fixative ensures the stability of diatom frustules due to the low pH of the solution. The preservative is present in the samples in a concentration of 2%. An alkaline preservative will tend to allow silicates associated with cell wall structure to go into solution resulting in cells disappearing from the samples over time (Sournia, 1978). Although the Lugol's is light sensitive, unlike Formalin Acetic Acid (FAA) which has a longer shelf life, the storage of samples in opaque bottles reduces the sample exposure to light. The Lugol's does provide some degree of staining for cell material enhancing the visual detection of cells. The wide spread usage of Lugol's as a preservative world wide also permits some limited comparison of sample collection protocols and identifications over a wider geographical range. It should also be noted that many preservatives, including those mentioned above may generate cell distortions in the case of unarmoured dinoflagellates, and in fact the disappearance of cell components in the case of coccolithophores. There are limitations to all of the preservation methods available for use.

Processing of Phytoplankton Samples

Phytoplankton samples will be processed for phytoplankton species identification and enumeration using microscopic methodology. A standard phase contrast microscope equipped with oil immersion capability will be used for observations at a variety of magnifications (ie. 10X, 40X, 100X).

Water samples containing phytoplankton will be prepared for microscopical examination using settling chambers. Phytoplankton settling chambers were first described by Utermohl (1958) and later by Hasle (1978) and others. The fundamental technique allows the placement of a known sample volume over a settling site, a glass cover slip or specialised microscope slide, for a given period of time. The samples generally settle overnight and the water column is then carefully removed leaving the cells on the sample site to be observed on an inverted microscope.

Settling Chamber (SC) preparations

Phytoplankton samples can be concentrated onto standard microscope slides using phytoplankton settling chambers (SC method) originally described by Knoechel and Kalff (1976) and Crumpton and Wetzel (1981). This method allows the examination of the microscope slide on any standard light microscope and avoids the use of specialised inverted microscopes. The settling chamber (SC) method may take as long as four days due to the time needed for very small particles in the 1-2 µm range to settle

on the slide (Pauley, 1987). An advantage of the SC method is that, given enough time, virtually every particle in the sample will settle to the bottom of the chamber.

This settling chamber method allows the settling of both marine and freshwater phytoplankton specimens onto standard microscope slide for prolonged storage and later examination. Pre-labelled glass slides are installed between the top and bottom plates of the settling chamber. A pre-determined, well mixed, volume of preserved sample is pipetted into a settling chamber tower that has been placed on the settling chamber. Installing taller towers allows larger volumes to be settled. Aliquots that do not fill the tower may have filtered seawater/ distilled water added to ensure that the tower's capacity is complete. A coverslip is then placed over the settling chamber tower to prevent leakage of the contents. The tower is left in the settling position for at least 24 hours, dependent on the particle size being examined, and then the remaining water is dumped prior to rinsing the sample to remove excess salt. The rinse is left for 24 hours to settle any resuspended particles and then removed and the slide is allowed to dry. The slide is then removed from the settling chamber and a cover slip is mounted on the sample area with a drop of mounting media (usually pre-filtered 2% Lugol's seawater solution). The sides of the coverslip can be sealed with clear nail polish and the slides stored for use and re-use for an extended period of time.

In a correctly settled slide, because of its random particle distribution, cells can be enumerated using volumetrically corrected field counts for those cell densities too high to count the entire sample; that is, it is not always necessary to count all the cells of interest on a settled slide. The diameter of the field of magnification used is representative of a specific volume of water (a sub-column of water within the settling tower); i.e., the area of the preparation in view subtends a small cylinder of the sample water whose volume can be calculated. The volume of water viewed for enumeration of a particular field can be expressed in the following equation:

$$v = \pi \cdot r^2 \cdot h$$

where: v = volume of the cylinder of water overlying the area viewed

$\pi = 3.1415$

r^2 = the square of the radius of field of view in μm^2

h = height of cylinder viewed in μm

To make an example calculation, let us assume that all the cells in 10 randomly selected fields have been counted and added together and that the sum of the field counts is 250; the average field count, n, therefore, is 25 cells$\cdot$(field)$^{-1}$.

Next, assume that, using a particular objective lens (say 20x), that the diameter of a field of view as measured by the grid or scale in the eyepiece is 985 μm. The radius (r) of this field is 492.5 μm and, therefore, $r^2 = 2.426 \cdot 10^5$ μm^2.

The height of the cylinder viewed is actually calculated from the measured volume of the settling chamber tower (this is done gravimetrically) and the nominal diameter of the acrylic tubing used to make the tower. In this case, assume that we have a settling chamber whose volume is 10.2 ml and whose height $h = 4.936 \cdot 10^4$ μm.

Therefore the volume of the cylinder overlying a typical field of view may be calculated as

$$v = \pi \cdot 2.425 \cdot 10^5 \ \mu m^2 \cdot 4.936 \cdot 10^4 \ \mu m$$

or

$$v = 3.760 \cdot 10^{10} \ \mu m^3 \cdot (field)^{-1}$$

or, since there are 10^{12} μm^3 in 1 ml,

$$v = 0.0376 \text{ ml·(field)}^{-1}.$$

The reciprocal of this is 26.596 fields·ml^{-1}; this is k, a factor we use to calculate N, the number of cells·ml^{-1}. Thus

$$N = n \cdot k$$

or

$$N = 25 \text{ cells·(field)}^{-1} \cdot 26.596 \text{ fields·ml}^{-1}$$

i.e.,

$$N = 664.89 \text{ cells·ml}^{-1}.$$

To convert to cells·L^{-1}, multiply by 1,000; there were, therefore, 664,890 cells·L^{-1} in the sample.

Taxonomic Identifications

Efforts will be made to identify all phytoplankton species present. This is always a daunting task complicated by morphological variations within species, preservation artefacts, magnification limitations of the light microscope and the presence of species not as yet identified at all. Extensive literature does however exist for a great deal of the species that will be encountered such as primary reference material associated with the local geographical area, ie. Berard-Therriault et al. (1999), as well as a multitude of other reference sources (see Taxonomic Reference List).

Cells will be identified to genus and species if possible using light microscopy. The possibility does exist to examine phytoplankton specimens at the sub-microscopic level using electron microscopy. It may be important in some cases, particularly when presence of potentially harmful algal species is concerned, that identifications are confirmed or verified as they may make a unique contribution to the phytoplankton assemblage.

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Appendix IV. Example of phytoplankton count report

Sample #	Sample ID	Location	Station	Date	Depth	Group	Genus	Species	Cells/5ml	Cells/L
1	186884	Labrador Sea	L3-26	05/21/97	10	Diatom	*Chaetoceros*	*fragments*	3809	761800
1	186884	Labrador Sea	L3-26	05/21/97	10	Diatom	*Thalassiosira*	*spp.*	1488	297600
1	186884	Labrador Sea	L3-26	05/21/97	10	Diatom	*Pseudonitzschia*	*delicatissima*	124	24800
1	186884	Labrador Sea	L3-26	05/21/97	10	Diatom	*Fragilaria*	*sp.*	60	12000
1	186884	Labrador Sea	L3-26	05/21/97	10	Diatom	*Amphiporora*	*sp.*	56	11200
1	186884	Labrador Sea	L3-26	05/21/97	10	Diatom	*Fragilaria*	*oceania*	1777	355400
1	186884	Labrador Sea	L3-26	05/21/97	10	Diatom	*Fragilaria*	*nana*	1901	380200
1	186884	Labrador Sea	L3-26	05/21/97	10	Diatom	*Eucampia*	*groenlandica*	728	145600
1	186884	Labrador Sea	L3-26	05/21/97	10	Dinoflagellate	*Prorocentrum*	*balticum*	178	35600
1	186884	Labrador Sea	L3-26	05/21/97	10	Dinoflagellate	*Gyrodinum*	*sp.*	181	36200
1	186884	Labrador Sea	L3-26	05/21/97	10	Other	*Cryptomonad*	*sp.?*	178	35600
1	186884	Labrador Sea	L3-26	05/21/97	10	Other	*Unidentified*	*flagellates*	1724	344800
1	186884	Labrador Sea	L3-26	05/21/97	10	Other	*Phaeocyctis*	*pouchetii*	8917	1783400
2	186921	Labrador Sea	L3-25	05/21/97	10	Diatom	*Thalassiosira*	*spp.*	1963	392600
2	186921	Labrador Sea	L3-25	05/21/97	10	Diatom	*Pseudonitzschia*	*delicatissima*	399	79800
2	186921	Labrador Sea	L3-25	05/21/97	10	Diatom	*Rhizosolenia*	*fragments*	49	9800
2	186921	Labrador Sea	L3-25	05/21/97	10	Diatom	*Fragilaria*	*oceania*	1361	272200
2	186921	Labrador Sea	L3-25	05/21/97	10	Diatom	*Fragilaria*	*nana*	100	20000
2	186921	Labrador Sea	L3-25	05/21/97	10	Diatom	*Chaetoceros*	*concavicorne*	151	30200
2	186921	Labrador Sea	L3-25	05/21/97	10	Diatom	*Chaetoceros*	*fragments*	5343	1068600
2	186921	Labrador Sea	L3-25	05/21/97	10	Dinoflagellate	*Gyrodinium*	*sp.*	50	10000
2	186921	Labrador Sea	L3-25	05/21/97	10	Silicoflagellate	*Dictyocha*	*speculum*	52	10400
2	186921	Labrador Sea	L3-25	05/21/97	10	Other	*Phaeocystis*	*pouchetii*	26679	5335800
2	186921	Labrador Sea	L3-25	05/21/97	10	Other	*Unidentified*	*flagellates*	2930	586000
2	186921	Labrador Sea	L3-25	05/21/97	10	Other	*Cryptomonad*	*sp.?*	554	110800

Appendix V. Example of zooplankton sample analysis contract

I. Project description

The zooplankton samples are collected as part of the monitoring program for coastal waters of eastern Canada. The objectives of the program are to measure seasonal and interannual variability of biological and physical variables at fixed and transect stations. The particular objectives for the zooplankton component of the program are 1) to measure total biomass (g dw m^{-2}) and 2) to determine the zooplankton species abundances. The intent of the analysis is to measure levels of the most important taxa (in terms of numbers and/or biomass), rather than to resolve all constituents.

The samples are routinely collected with a 202 µm mesh ring net towed vertically from either the bottom or 1000 m (whichever is shallower) to the surface.

II. Sample analysis

1. Summary

- All organisms larger than 1 cm are identified, measured, weighted and then preserved anew;
- half the sample is used for dry weight measurement;
- half the sample is used for abundance/composition determination and then preserved anew.

2. Estimation of biomass

Accurately separating samples into size fractions has frequently proven to be very difficult because of the high concentrations of phytoplankton, appendicularians, jellies, salps, etc. This results in unreliable measurements. Therefore in order to reduce the difficulty of separating large and small plankton for biomass measurements, the following protocol is to be used. After pouring off the formalin, all organisms larger than 1 cm are manually separated out. These organisms are identified according to the criteria listed in 3c (as for small organisms) and weighed (wet weights, which permits these organisms to be preserved). This total weight is reported. The remainder of the sample (i.e. all organisms less than 1 cm) is split once using a Matoda splitter. One half of the sample is used for dry weight where the animals are collected on a pre-weighed shark skin filter, dried at 60 °C for 48 hours and weighed. The other half is used for abundance/composition determinations as discussed below.

3. Abundance and composition

The second split is used to estimate zooplankton abundance and composition. The subsampling methodology must be one of the techniques described in Van Guelpen et al. (1982). The "bulb pipette" technique, however, is unacceptable. Subsamples are such that a minimum of 200 organisms per sample are counted and identified according to criteria a - c. Once the 200 organism count has been obtained, additional aliquots shall be taken until approximately 75-100 Calanus spp have been identified and staged. If several stages and/or all species are present, a total of 150-200 Calanus should be counted.

a. Copepods are to be identified to species whenever possible. *Pseudocalanus* should be identified as *Pseudocalanus* spp.

b. All developmental stages of *Calanus finmarchicus, Calanus glacialis* and *Calanus hyperboreus* copepodites are to be identified.

c. All other zooplankton are to be classified according to the following taxonomic categories:

Amphipods (genus)
Bivalves
Chaetognaths (genus)
Coelenterates (genus where possible)
Ctenophores (genus)

Cladocerans (genus)
Decapods (adults: genus; larvae: group)
Echinoderms (larvae, juvenile)
Euphausiids (species)
Fish eggs (species)
Fish larvae (species)
Larvaceans (genus)
Mysids
Ostracods
Polychaetes
Pteropods

If a taxon not listed is encountered, the level of identification will be established after consultation with the scientific authority.

4. DFO Scientific Authorities

Dr. Pierre Pepin
Dr. Doug Sameoto
Dr. Michel Harvey

5. References

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